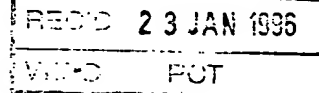




6 PCT/AU 95/00875  
08/849543



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**PRIORITY DOCUMENT**

I, MARCIA PATRICIA BAKER, ACTING ASSISTANT DIRECTOR PATENT SERVICES, hereby certify that the annexed is a true copy of the Provisional specification as filed on 22 December 1994 in connection with Application No. PN 0307 for a patent by ARUBA INTERNATIONAL PTY LTD filed on 22 December 1994.

I further certify that the annexed specification is not, as yet, open to public inspection.

WITNESS my hand this Third  
day of January 1996

MARCIA PATRICIA BAKER  
ACTING ASSISTANT DIRECTOR PATENT  
SERVICES



AUSTRALIAN	
PROVISIONAL No.	DATE OF FILING
PN0307	22 DEC. 94
PATENT OFFICE	

ARUBA INTERNATIONAL PTY LTD

AUSTRALIA  
Patents Act 1990

PROVISIONAL SPECIFICATION FOR THE INVENTION ENTITLED:

DISCONTINUOUS PLASMA OR SERUM DELIPIDATION

The invention is described in the following statement.

THIS INVENTION relates to plasma or serum delipidation in animals (which term shall indicate humans). In particular, it is directed to the removal of cholesterol, triglycerides and other lipids from the blood plasma or serum of such animals.

Cardiovascular diseases are responsible for a significant number of deaths in most industrialized countries.

One such disease is atherosclerosis which is characterised by local fatty thickening in the inner aspects of large vessels supplying blood to the heart, brain and other vital organs. These lesions obstruct the lumen of the vessel and result in ischaemia of the tissue supplied by the vessel. Prolonged or sudden ischaemia may result in a clinical heart attack or stroke from which the patient may or may not recover.

The relationship between dietary lipid, serum cholesterol and atherosclerosis has long been recognised. In many epidemiological studies it has been shown that a single measurement of serum cholesterol has proved to be a significant predictor of the occurrence of coronary heart disease.

Thus diet is the basic element of all therapy for hyperlipidaemia (excessive amount of fat in plasma). However, the use of diet as a primary mode of therapy requires a major effort on the part of physicians, nutritionists, dietitians and other health professionals.

If dietary modification is unsuccessful, drug therapy is an alternative. Several drugs, used singly or in

combination, are available. However, there is no direct evidence that any cholesterol-lowering drug can be safely administered over an extended period.

5 A combination of both drug and diet may be required to reduce the concentration of plasma lipids. Hypolipidaemic drugs are therefore used as a supplement to dietary control.

10 Many drugs are effective in reducing blood lipids, but none work in all types of hyperlipidaemia and they all have undesirable side effects. There is no conclusive evidence that hypolipidaemic drugs can cause regression of atherosclerosis.

15 In view of the above, new approaches have been sought to reduce the amount of lipid in the plasma of homozygotes and that of heterozygotes for whom oral drugs are not effective.

20 Plasmapheresis (plasma exchange) therapy has been developed and involves replacement of the patient's plasma with donor plasma or more usually a plasma protein fraction. This treatment can result in complications due to the possible introduction of foreign proteins and transmission of infectious diseases. Further, plasma exchange removes all the plasma proteins as well as very low density lipoprotein (VLDL), low density lipoprotein (LDL), and high density lipoprotein (HDL).

30 It is known that HDL is inversely correlated with the severity of coronary arterial lesions as well as with the likelihood that these will progress. Therefore, removal of HDL is not advantageous.

Known techniques also exist which can totally remove LDL from plasma. These techniques include absorption of LDL in heparinagarose beads (affinity chromatography) or the use of immobilised LDL-antibodies. Other methods  
 5 presently available for the removal of LDL involve cascade filtration absorption to immobilised dextran sulphate and LDL precipitation at low pH in the presence of heparin. Each method specifically removes LDL but not HDL.

- 10 LDL aphaeresis has, however, disadvantages. Significant amounts of other plasma proteins are removed during aphaeresis and to obtain a sustained reduction in LDL-cholesterol, LDL aphaeresis must be performed frequently (up to once weekly). Furthermore, LDL  
 15 removal may be counter productive; low blood LDL levels will result in increased cellular cholesterol synthesis.

To satisfy the need for a method of achieving a reduction in plasma cholesterol, and in particular LDL-cholesterol, in homozygous familial  
 20 hypercholesterolemia and heterozygous familial hypercholesterolemia patients other than by diet and/or drug therapy, an extra corporeal lipid elimination process, termed "cholesterol aphaeresis", has been developed. In cholesterol aphaeresis, blood is  
 25 withdrawn from a subject, plasma separated from the blood and mixed with a solvent mixture which extracts lipid from the plasma, after which the delipidated plasma is recombined with the blood cells and returned to the subject.

- 30 The advantage of this procedure is that LDL and HDL are not removed from the plasma but only cholesterol, some

phospholipids and triglycerides are removed. United States Patent No 4,895,558 describes such a system.

While cholesterol aphaeresis has overcome the shortcomings of dietary and/or drug treatments and other  
5 aphaeretic techniques, existing apparatus for cholesterol aphaeresis does not provide a sufficiently rapid process. For use in a clinical setting, apparatus is required which effects delipidation more efficiently. Furthermore, flow rates of the order of 70  
10 ml/min are required for cholesterol aphaeresis of a human subject.

Thus the cholesterol aphaeresis described in the aforementioned US Patent No 4,895,558 was improved by incorporating into the system a spinner to disperse the  
15 incoming plasma laterally into the extracting solvent in the form of fine droplets to improve separation efficiency. This improved system is described in International Patent Application No PCT/AU94/00415.

Unfortunately, practice has established that the  
20 cholesterol aphaeresis systems described above still suffer from a number of disadvantages.

The first disadvantage is the explosive nature of the solvents used to delipidate this plasma. These solvents are, by the very nature of the continuous  
25 systems, in close proximity to the patient and medical staff. This hazard is clearly present for the duration of the delipidation process which usually runs for several hours.

The second disadvantage is that, to date, a reliable  
30 procedure is not available to remove totally all of the

solvents used in the delipidation before the treated plasma is returned to the patient.

In particular, the use of the preferred solvent 1-butanol in the delipidation is of concern as it can now  
5 be established that that solvent can be present as 1% to 5% of the treated plasma that is returned to the patient. This is because continuous systems can only include a single wash to remove solvents such as 1-butanol and that single wash is now found to be  
10 insufficient. It is not possible to provide sequential multi-washes in a continuous system because the patient would have to supply an unacceptable volume of blood to maintain each stage of the system overall.

The long term toxicity of 1-butanol is not known,  
15 especially when directly present in the blood stream- it may cross the blood brain barrier. Certainly, external contact with this solvent is known to cause irritation of mucous membranes, contact dermatitis, headaches, dizziness and drowsiness.

20 A third disadvantage is that the continuous systems described above are not suitable for the delipidation of serum. If serum can be delipidated, there would be the advantage of favourably altering the blood rheology in that the viscosity will decrease following delipidation  
25 resulting in better haemodynamics for the originally impaired blood circulation.

Yet a fourth disadvantage is that delipidation in a continuous system is undertaken over several hours. Apart from the prolonged exposure to the hazardous  
30 solvents as discussed above, the equipment and staff are committed to a single patient. As the removal of plasma

or other blood fractions and their subsequent return to the patient as individual steps each only take a few minutes, it would be advantageous if the relatively lengthy delipidation step could be undertaken off site, thus freeing the patient, medical staff and equipment for other matters.

It is thus an object of the present invention to overcome, or at least ameliorate, the above-mentioned disadvantages in the provision of a method for delipidating not only plasma but also serum and other blood fractions which substantially reduces the exposure of the patient to the potentially hazardous solvents used, which also can effectively remove all traces of solvent(s) used in that delipidation, and which significantly reduces the contact time between the patient and the actual delipidation process.

As a first aspect of the present invention, there is provided a method for the removal of cholesterol, triglycerides and other lipids from animal plasma, serum, or other suitable blood fractions, as a discontinuous flow system, said method comprising withdrawing blood from a subject, separating the required fraction from the blood and mixing with a solvent mixture which extracts the said lipids from the fraction, after which the delipidated fraction is recombined with the blood cells and returned to the subject, characterised in that the solvent extraction step is carried out separately and remote from the subject.

Preferably, as part of the solvent extraction step, the resultant delipidated fraction-containing phase is washed with another solvent, preferably diethyl ether,

to remove substantially all of the original solvent before the blood fraction is returned to the patient.

More preferably, four (4) washes are undertaken.

In a second aspect of the present invention, the solvent  
5 extract step comprises:

- (a) mixing the solvent mixture containing the fraction  
with glass beads, said glass beads being of a  
density substantially mid-way between the density  
of the fraction and the density of the solvent  
10 mixture;
- (b) isolating the thus delipidated fraction- containing  
phase; and
- (c) mixing the delipidated fraction with sintered  
spheres, said spheres being porous and containing  
15 within the pores an absorbent specific for the  
solvent that is being removed.

Preferably, to obtain a density substantially mid-way  
between the density of the fraction and the density of  
the solvent mixture, the glass beads contain entrapped  
20 air.

More preferably, as the density of plasma is  
approximately 1.006 g/ml and the solvents used generally  
have a density of approximately 0.8 g/ml, the density of  
the glass beads will be around 0.9 g/ml.

25 Preferably, the sintered spheres will be approximately 2  
to 5 mm in diameter with the pores of the spheres being  
less than 50 Å in diameter.

Preferably, the absorbents used in the sintered spheres are the macroporous polymeric beads for absorbing organic molecules from aqueous solutions marketed by Bio-Rad Laboratories under the trade name Bio-Beads SM.

- 5 If the solvent used to delipidate the fraction is 1-butanol, then the absorbent is preferably Bio-Beads SM-2.

- 10 Preferably, as part of isolating the delipidated fraction-containing phase, that phase is subsequently washed with another solvent, preferably diethyl ether, to remove a substantial amount of the original solvent before the treatment with the spheres.

More preferably, that phase is washed at least three (3) times.

- 15 The plasma may be human plasma or plasma from other living animals. The plasma can be obtained from human or animal blood by known plasma separating techniques which include centrifugal separation, filtration and the like.
- 20 Similarly, the serum or other lipid-containing fraction can be derived from human or other living animals by known techniques.

- 25 Suitable solvents for the extraction comprise mixtures of hydrocarbons, ethers and alcohols. Preferred solvents are mixtures of lower alcohols with lower ethers. The lower alcohols suitably include those which are not appreciably miscible with the plasma and these can include the butanols (butan-1-ol and butan-2-ol). C1-4 ethers are also preferred and these can

include the propyl ethers (di-isopropyl ether, propyl ether). Other solvents which may be applicable can include amines, esters, hydrocarbons and mixtures providing that the solvent can (1) rapidly and  
5 preferably remove cholesterol from the plasma, (2) is substantially immiscible with the plasma, (3) can be removed from the plasma, and (4) does not denature the desired moieties. Preferred solvent compositions are butanol with di-isopropyl ether and these may be in the  
10 ratio of 0% - 40% of the alcohol with 100% - 60% of the ether.

In one embodiment of the present invention, the continuous flow system described in US Patent No 4,895,558 is modified to a discontinuous system by  
15 removing the appropriate blood volume to be treated and subjecting that volume to delipidation at a site remote from the patient. The patient is fed with an appropriate replacement fluid, if necessary, before the treated volume is subsequently returned to that patient.

20 In a second embodiment of the present invention, the continuous flow system described in International Patent Application No PCT/AU94/00415 is modified to a discontinuous system by removing the appropriate blood volume to be a site remote from the patient before the  
25 plasma is dispersed into small droplets into the solvent by the dispersing means. Once again, the patient is fed with an appropriate replacement fluid, if necessary, before the treated volume is subsequently returned to that patient.

30 In either of the above embodiments, the extraction step can include, in accordance with the present invention,

either multiple washing of the extracted phase and/or using the glass beads and sintered spheres.

By adapting the prior art methods to discontinuous flow systems, the present invention can remove or at least  
5 significantly reduce any danger to patients and medical staff from the explosive nature of the solvents employed.

Further, by using the improved solvent extraction methods of the present invention, all of the potentially  
10 poisonous extraction solvents can be removed before the treated blood is returned to the patient.

Also, the improved solvent extraction method of the present invention is not limited to plasma delipidation but also it is applicable to the delipidation of serum,  
15 thus providing advantageous changes to the blood rheology of the originally impaired blood circulation of the patient.

Finally, as the present invention is a discontinuous system, it is not essential to return the delipidated  
20 blood fraction immediately to the patient. If necessary, reintroduction of the delipidated fraction can occur up to two (2) weeks after it was first removed from the patient. This option leads to particular advantages such as, economies of scale when several  
25 patients have to be treated simultaneously, the freeing of medical staff and equipment for other duties, and the reduction in stress for the patient whom no longer has to be hooked up to a delipidation apparatus for several continuous hours.

The embodiments are described by way of illustrative examples only and various changes and modifications may be made thereto without departing from the inventive concept.

5 DATED this twenty second day of December 1994

ARUBA INTERNATIONAL PTY LTD  
By its Patent Attorneys  
GRANT ADAMS & COMPANY